Supporting Information

A synthetic biosensor for detecting putrescine in beef samples

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Supplementary Information

Protocol S1: Preparation of cell lysate for cell-free protein synthesis reactions

To prepare the cell lysate for cell-free protein synthesis reactions (CFPS), we obtained the protocol from but made modifications to the protocol to fit our application.[1, 2] *E. coli* BL21Star™ (DE3) cells were acquired from a glycerol stock, streaked on an LB agar plate, and incubated overnight at 37 °C. A single colony of *E. coli* BL21Star™(DE3) was inoculated into a starter culture of 50 mL in LB with no selection, and grown overnight (15-18 h) at 37 °C and 200 rpm. A solution of 2x YTP was prepared by dissolving 5.0 g sodium chloride, 16.0 g of tryptone, 10.0 g of yeast extract, 7.0 g of potassium phosphate dibasic, and 3.0 g of potassium phosphate monobasic into 750 mL of ddH_2O. A glucose solution was prepared by dissolving 250 mL of ddH_2O with 18 g of D-glucose. 2xYTP was transferred to a 2 L baffled flask and autoclaved for 30 min at 121 °C. The glucose solution was filter sterilized using a 0.22 μm vacuum filtration system. On the day of inoculation, the glucose solution was added to the 2xYTP media and warmed in a 37 °C incubator. Overnight starter culture of BL21star™ (DE3) was removed from shaking incubator and diluted in a 1:10 ratio and OD_{600} was measured using a spectrophotometer. Overnight starter culture was added to 1L of 2xYTPG media and inoculated culture was incubated at 37 °C while shaking at 200 rpm. OD_{600} was monitored every hour to ensure stable growth until culture reached mid exponential phase or OD_{600} = 0.6. After reaching this OD, the culture was induced with 1 mL of 1 M isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. After induction, the OD_{600} of the culture was monitored every 20-30 min and was immediately immersed in an ice-water bath to arrest growth at OD_{600} 1.9-2.0. The culture was transferred into cold 1 L centrifuge bottles (Beckman Coulter, Indianapolis, IN) and centrifuged for 10 min at 5000 x g and 4°C (Avanti® J-E Centrifuge, Beckman Coulter, Indianapolis, IN). Supernatant was removed, and

the pellet was placed on ice using a sterile spatula and transferred to a cold 50 mL Falcon tube placed on ice. Cells were washed three times with 30 mL of cold S30 buffer (14 mM Mg $(OAc)_2$, 10 mM Tris (OAc), 60 mM KOAc, 2 mM dithiothreitol) by resuspension via vortexing with short bursts (20 - 30 s) with rest periods (1 min) in between. For each wash, the suspension was centrifuged at 5000 x g and 4°C for 10 minutes. After disposing the supernatant, the pellet was weighed, then flash frozen via liquid nitrogen and kept at – 80 °C until extract preparation. The frozen cell pellet was combined with 0.8 mL of S30 buffer per 1 gram of cell pellet and thawed on ice. Once the cell pellet was fully thawed, it was resuspended via vortexing, with short bursts (20 - 30 s) and rest periods (1 min), until no visible clumps were observed. 1 mL of resuspended cell pellet was transferred to 1.5 ml Eppendorf tubes. A Q125 sonicator (Qsonica, Newtown, CT) with a 3.175 mm probe was used at a frequency of 20 kHz and resuspension was sonicated for 45 s followed by 59 s off for 3 total cycles, with amplitude set at 50 %. Immediately after, 4.5 µL of 1 M DTT was added to the lysate, Eppendorf tubes were closed and gently inverted 3-4 times. In total, 800-900 J of energy was delivered to each 1.5 mL microfuge tube containing 1mL of resuspended cells. Using a Pierce™ BCA assay kit (ThermoScientific), total protein concentration was measured of the lysate. To maintain consistency between batches, cell-extract was concentrated using a 10,000 MWCO centrifuge column (GE Healthcare; Vivaspin20) to obtain a final concentration of 15mg/ml. The lysate was then centrifuged using a tabletop centrifuge at 14,000 × g and 4 °C for 15 minutes. Following centrifugation, the supernatant (or lysate) was pipetted into new and sterile 1.5 mL Eppendorf tubes, flash frozen in liquid nitrogen, and kept in a – 80 °C freezer until use.

Protocol S2: Quantification of the Reporter Protein

Using a half area 96-well black polystyrene plate (Greiner, Germany), 48 µL of 0.05 M HEPES (pH 8) was loaded for each sample and replicates. Reactions are removed from 30ºC incubator for endpoint measurement, and 2 µL were transferred into the 48 µL of 0.05 M HEPES, pH 8. Samples were pipetted up and down again in the well to mix. Once all reactions are loaded, the 96 well plate was placed in the ClARIOstar and the fluorescence was measured at an excitation wavelength of 488nm and an emission wavelength of 507nm using a fluorescence microplate reader (CLARIOstar, BMG Labtech, Germany). Using an eGFP standard curve, the concentration of eGFP can be calculated from the obtained fluorescence readings.

Protocol S3: Methods for Preparation of an eGFP Standard Curve

A standard curve of quantitation of eGFP was prepared in the following manner. From a glycerol stock of BL21(DE3) star™-eGFP was streaked on an agar plate with 100µg/ml carbenicillin selection and incubated at 37ºC overnight. A single colony was inoculated in 10ml of LB with 50 µg/ml carbenicillin in a round bottom test tube and grown over night at 37 °C and 250 rpm. 5ml culture was transferred to a 15ml falcon tube and centrifuged at 10,000 x g at 4ºC for 5 min. Supernatant was discarded, his-tag purification was carried out using HisPur™ cobalt resin (Invitrogen). Pellet was immersed in ice and 800 µl of wash buffer was added and was resuspended by pipetting up and down. Pellet was incubated on ice for 20-30 min, resuspension solution was transferred to 1.5ml microcentrifuge tubes and sonicated for 10s on, 10 s off, until the total J delivered was ~200 J. Sample was centrifuged at 10,000 x g for 5 min at 4ºC, supernatant was separated and collected in separate 1.5 mL microfuge tube. Cobalt purification columns were prepared by allowing the storage buffer to drip through and equilibrated the column with 2 column bed volumes of wash buffer (400 µL). 400µL of supernatant was transferred to spin column and

bottom was plugged to prevent leakage and inverted six times. Spin column was placed on ice and ice box was placed on orbital shaker and incubated for 30 minutes. Bottom plug was removed, and spin column was centrifuged at 700 × g for 2 minutes at 4°C to remove supernatant. Process was repeated again with the rest of 400µL of supernatant. The spin column was washed with two resin beds of wash buffer (400 µL) and centrifuged at 700 × g for 2 minutes at 4°C and fractions were collected in a microcentrifuge tube. The spin column was washed three times, and each fraction was collected. Elution was performed by adding 2 resin beds of elution buffer (400 µL) and centrifuged at 700 × g for 2 minutes at 4°C and fractions were collected in a microcentrifuge tube. Proteins were eluted from the spin column two more times and fractions were collected each time. The spin column was regenerated by washing with 10 resin bed volumes of MES buffer (20mM 2-(N-morpholine)-ethane sulfonic acid, 0.1M sodium chloride; pH 5.0) then centrifuging at 700 × g for 2 minutes at 4°C. Then washing the spin column with 10 resin bed volumes of ultrapure water and centrifuged at 700 × g for 2 minutes at 4°C. The cobalt resin was stored as 50% slurry and 20% ethanol and stored at 4°C. To remove imidazole from the purified protein dialysis was performed using Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes (Invitrogen) against dialysis buffer (150 m*M* NaCl, 100 m*M* HEPES–NaOH, pH 8.0) and concentrated using Amicon Ultra-15 concentrators (Millipore, Billerica, MA). Using Pierce BCA protein assay kit, the overall concentration of the purified protein was determined by measuring absorbance at 562nm, and extrapolating concentration from BCA standard curve. Serial dilutions of stock eGFP protein, ranging from 0 to 800µg/mL of protein was prepared in triplicates and was measured at 562nm to determine absorbance. Resulting measurements were used to plot a standard curve in order to convert fluorescence readings to concentration of eGFP in µg/mL as shown in **Figure S10**.

Protocol S4: Generating Golden Gate destination vector for repressor plasmid

BsaI restriction sites were inserted into the pFAB4876 backbone by performing two cycles of PCR. Primers were designed to incorporate the BsaI site into the 5´end of the forward primer while the reverse primer anneals back-to-back with the 5´end of the complementary region of the forward primer, refer to **Figure S2**. In the first cycle of PCR, the primer pair pFAB4876_frwd_BsaI and pFAB4876_rev_BsaI (Table 1) were used in a 50 µl PCR reaction containing 43.5µl G/C master mix, 2.5µl forward, 2.5µl reverse primer, 1.0µl of template, and 0.5µl Phusion® high-fidelity DNA polymerase (NEB) and was mixed by pipetting up and down. First round of PCR conditions are listed in **(Supplementary Table 5)**. Methylated template DNA was removed after PCR by restriction digest using DpnI (NEB) and purified using Invitrogen PCR purification kit. From the PCR reaction 5 µL of the template was mixed with 50 µL of chemically competent *E. coli* DH5α cells using heat shock transformation. After recovery in 200 µL of SOC medium, 100 µL of cells were plated onto LB plates with 50 µg/ml kanamycin and grown at 37 °C overnight. Five randomly selected colonies were grown in 5 mL of LB broth with 50 µg/ml kanamycin and grown over night in 37ºC shaking incubator at 250 RPM. Overnight culture was used to isolate the plasmid using Biobasic miniprep kit. Isolated plasmids were digested in a 50µL reaction consisting of 1 µg of plasmid template, 5 µL of cut-smart Buffer®, 20 U of BsaI-HF®, and topped up to 50 µL with nuclease-free water. Digest reaction was left to incubate at room temperature ~ 23 °C for 30 min and was then visualized on a 0.8% agarose gel. Plasmid with successful BsaI integration was used as a template for the integration of the second BsaI site.

The isolated plasmid was used as the template for the second round of PCR. In the second round of PCR, primers pFAB4876_frwd_2BsaI and pFAB4876_rev_2BsaI were used to introduce the second BsaI site; 5'-GGTCTC-3' into the pFAB4876 backbone. PCR reaction was performed

in 50 µl PCR using the same volumes and conditions as mentioned in the first round of PCR. The PCR reaction was purified using Invitrogen PCR purification kit then treated with DpnI (NEB) to remove methylated DNA template. 5µl of plasmid was transformed in 50µl of DH5α using heat shock transformation at 42°C for 30 seconds, and recovered with 200 µL of SOC media, and plated on pre-warmed 50 µg/ml kanamycin agar plate. Five randomly selected colonies were chosen and inoculated in 5 ml of LB broth with 50 µg/ml kanamycin and grown over night in 37°C shaking incubator at 250 RPM. Overnight culture was used to isolate the plasmid using Biobasic miniprep kit. Isolated plasmids were digested in a 50µL reaction consisting of 1µg of plasmid template, 5µL of cut-smart Buffer®, 20U of BsaI-HF®, and topped up to 50µL with nuclease-free water. Digest reaction was left to incubate at room temperature for 30 min and was then visualized on a 0.8% agarose gel. Plasmid with successful integration of second BsaI site was stored in 50% (v/v) glycerol solution for long term storage in -80°C freezer. The successful integration for two BsaI sites in pFAB4876 makes the plasmid golden gate compatible and was renamed pFAB.

Protocol S5: Escherichia coli strains, primers, plasmids, and reagents

All general-use reagents were purchased from Sigma, unless specified otherwise. Strains and plasmids were described in **Table S1**. Briefly, host strain *E. coli* DH5α and One Shot® BL21 Star™ were obtained from Invitrogen Corporation (Carlsbad, CA, USA). Competent DH5α and One Shot® BL21 Star™ were prepared using Hanahan method.[3] Unless otherwise stated, cells were cultured in lysogeny broth (LB) medium or M9 minimal media, with appropriate antibiotics (kanamycin, carbenicillin, or chloramphenicol at 50, 100, 35 µg/ml, respectively). All primers used were commercially synthesized by Biocorp (Montreal, QC, CA). The plasmid pFAB4876 was purchased from Addgene (#80649).[4] The plasmid pTU1 was obtained from the EcoFlex

MoClo kit purchased from Addgene (#1000000080). Phusion® high-fidelity DNA polymerase, Taq DNA polymerase, T4 DNA ligase, DpnI, and BasI-HF® were purchased from New England Biolabs (Ipswich, MA, USA). Invitrogen PCR purification kit, Biobasic gel extraction kit, and Biobasic miniprep kit were purchased from Cedarlane (Ontario, Canada).

Protocol S6: Construction of the putrescine biosensor: repressor and reporter plasmids

The putrescine biosensor contains a dual plasmid system consisting of a repressor (which we call 'PuuR') and a reporter plasmid. As shown in **Figure S1**, the repressor plasmid exclusively used parts from the Ecoflex MoClo kit[5] and was constructed using an optimized, one-pot Golden Gate assembly protocol adopted from previous work.[6] The repressor plasmid contained the modified pFAB back bone (see **Supplemental Protocol S1**), T7 promoter, RBS, His-tag, and terminator (BBa_B0015) genes and the repressor protein PuuR (see **Table S2** for DNA sequences) which was commercially synthesized by Twist Bioscience (San Francisco, CA, USA). For the repressor plasmid assembly, BsaI restriction site were used as the type IIS restriction cut site for Golden Gate assembly. The PuuR DNA insert was PCR amplified by primers PuuR_R and PuuR_F harboring BsaI restriction sites (see **Table S3** primer sequences). Inserting the restriction site was performed in a 50 µL PCR reaction containing 43.5 µL GC master mix (5x G/C buffer, 100% DMSO, 100 mM ATP/TTP/GTP/CTP), 2.5 µL of primer PuuR_R, 2.5 µL of primer PuuR_F, 1.0 µL of PuuR template, and 0.5 µL Phusion® high-fidelity DNA polymerase (NEB) using PCR conditions listed in (**Table S4)**. The PCR reaction of PuuR was then loaded onto a 0.8% agarose gel and purified using BioBasic gel extraction kit according to manufactures instructions. The concentration of the gel purified PuuR was measured using a Tecan Infinite® 200 PRO (Tecan Group Ltd., Switzerland) and was used for the Golden Gate reaction in the next step. To prepare for the assembly reaction, an overnight culture of the vector backbone pFAB was inoculated in LB

with kanamycin selection, and isolated following the manufacturer's instructions from the Biobasic miniprep kit. Additionally, T7 promoter, RBS-linker, His-tag, and terminator (BBa_B0015) genes from the pTU1 backbone were obtained from the EcoFlex MoClo kit (Addgene) and were each inoculated with 5 mL of LB media with 35 µg/ml of chloramphenicol selection and cultured overnight at 37°C. Plasmids containing each gene were isolated using the Biobasic miniprep kit. To perform the assembly, 1.5 µL 10× T4 Ligation Buffer, 1 µL BsaI (or BsaI-HF®) (20 units), 1 µL T4 DNA Ligase (1–3 units), 50 ng pFAB destination vector, and 50 ng of each part (T7 promoter, RBS-linker, His-tag, PuuR insert, and terminator (BBa_B0015)) were added into a tube and topped up to 15 µL with ddH_2O and mixed via pipetting up-and-down. The reaction was incubated in the thermal cycler with the following program: 37 °C for 5 min then 16 °C for 10 min—repeated for 30 cycles—with an ending cycle at 50 °C for 5 min and at 80 °C for 5 min. Next, 2.5 µL of the assembly reaction was added to 25 µL DH5α for heat-shock transformation at 42 °C for 45 seconds and recovered in 200 µL SOC media and plated on pre-warmed 50 µg/mL kanamycin agar plates. Five randomly chosen transformants were selected for colony PCR using Taq polymerase in a 50 µL reaction using primers pFAB_frwd and pFAB_rev, reaction was carried out following reagents and thermocycler conditions listed in **(Table S4).** Colonies with positive colony PCR results were inoculated overnight in LB with 50 µg/mL kanamycin. A glycerol stock was made by mixing 1 mL of overnight culture with 1mL of 50 % (v/v) glycerol solution in a cryovial tube and stored for long term storage in -80 °C freezer.

For the reporter plasmid, there were four parts that were assembled in the plasmid backbone – the *puuO* promoters, RBS, eGFP, and the terminator (**Table S2)**. In total, 11 synthetic promoters **(Table S2)** were built using primers by a sowing PCR method to build the library (see **Figure S2** for workflow). To assemble each synthetic promoter, we performed two rounds of PCR: in the

first round, the core promoter was amplified by two over lapping primers (primer_2F and primer_2R; **Table S3**). To add flanking BsaI restriction sites to the core promoter, a second round of PCR was performed using primers: primer_R and primer_F. Each PCR reaction contained a 50 µL reaction volume consisting of 0.5 µL Phusion® high-fidelity DNA polymerase (NEB) with 2.5 µL of each primer: primer_2F and primer_2R and the reaction was topped up with 43.5 µL G/C master mix (5x G/C buffer, 100% DMSO, 100 mM ATP/TTP/GTP/CTP). The conditions of the PCR followed **Table S4**. Gel purification of the promoters and overnight inoculations of the pTU1 backbone, RBS, eGFP, and terminator (BBa_B0015) and isolation followed the protocol for the repressor plasmid except with 35 µg/ml chloramphenicol selection for inoculation. In addition, assembly **(**using Golden Gate **Table S5)**, transformation, and colony PCR protocols followed the protocol for the repressor plasmid. Successful assemblies were inoculated overnight in 5 mL of LB with 100 µg/mL carbenicillin. To make a glycerol stock, 1 mL of overnight culture was mixed with 1 mL of 50% (v/v) glycerol solution in a cryovial tube for long term storage in -80 ˚C freezer.

Sequential transformation was used to incorporate both the repressor and reporter plasmids into BL21 DE3 *E.coli* for our cell-based sensors. One Shot® BL21 Star™ competent cells were prepared using Hanahan method.[3] To start, 50 µL of BL21 DE3 star™ competent cells were thawed on ice, and 10 ng of repressor plasmid (PuuR) was added to 50 µL of competent cells and gently mixed by up and down pipetting. For the first transformation, the mixture was incubated on ice for 30 min, heat shocked for 30 s at 42 °C, and returned to ice for 5 min. 950 µL of room temperature SOC media was added to reaction tube and incubated in shaking incubator for 1 h at 37 °C. After 1 h, reaction tubes were spun down and 900 µL of LB was removed. The remaining 100 µL was resuspended and spread on pre-warmed agar plates with 50 µg/ml of kanamycin selection and incubated overnight at 37 °C. Five randomly chosen colonies were selected for

colony PCR reaction using the same conditions as above. Successful transformants were inoculated overnight in 5 mL of LB with 50 µg/mL kanamycin to make a starter culture for competent cells. The 5 mL starter culture of BL21 DE3 star™ with repressor plasmid was used to prepare chemically competent cells according to the Hanahan method. Next, 50 µL of the competent cells containing the repressor plasmid was used to transform 10 ng of each reporter plasmid and was used for heat shock transformation. After applying 42 °C for 30 s, cells were plated on agar plates with 50 µg/mL of kanamycin and 100 µg/mL carbenicillin. Successful transformants were picked inoculated in LB media with both 50 µg/mL of kanamycin and 100 µg/mL carbenicillin and grown overnight. This was done for the promoter library, and 1 mL of overnight culture was mixed with 1 mL of 50 % (v/v) glycerol solution in a cryovial for long term storage in -80 °C freezer.

Table S1: *Escherichia coli* strains and plasmids used in this study

Strains/Plasmids	Description	Source/Reference
DH5α	F- ϕ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rk- mk+) phoA supE44 λ- thi-1 gyrA96 relA1	Invitrogen
One Shot® BL21 Star™	F- ompT hsdSB(rB- mB-) gal dcm rne131 (DE3)	Invitrogen
JM109	endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB$^+$ Δ(lac-proAB) e14- [F$'$ traD36 proAB$^+$ lacI^q lacZΔM15] hsdR17($r_K^-m_K^+$)	New England Biolabs
Plasmids		
pProm1_BCD1-GFP	Kan^R , p15A, sfGFP under constitutive promoter, 2-kb	Addgene (#80649)
pTU1	Amp^R , pBR322ori, 2-kb	Addgene (#72934)
pTU1_eGFP	Amp^R , pBR322ori,eGFP under constitutive T7 promoter, 3-kb	This study
pTU1_puuAp_egfp	Amp^R , pBR322ori,eGFP under PuuAp promoter, 3-kb	This study
pTU1_TacR(3)_egfp	Amp^R , pBR322ori,eGFP under TacR(3) promoter, 3-kb	
pTU1_TacR(2)_egfp	Amp^R , pBR322ori,eGFP under TacR(2) promoter, 3-kb	
pTU1_LR(2)_egfp	Amp^R , pBR322ori,eGFP under LR(2) promoter, 3-kb	
pTU1_P_{hyb}(1A)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(1A) promoter, 3-kb	This study
pTU1_P_{hyb}(2A)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(2A) promoter, 3-kb	This study
pTU1_P_{hyb}(3A)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(3A) promoter, 3-kb	This study
pTU1_P_{hyb}(1B)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(1B) promoter, 3-kb	This study
pTU1_P_{hyb}(2B)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(2B) promoter, 3-kb	This study
pTU1_P_{hyb}(3B)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(3B)) promoter, 3-kb	This study
pTU1_P_{hyb}(1C)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(1C) promoter, 3-kb	This study
pTU1_P_{hyb}(2C)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(2C) promoter, 3-kb	This study
pTU1_P_{hyb}(3C)_egfp	Amp^R , pBR322ori,eGFP under P_{hyb}(3C) promoter, 3-kb	This study
pFAB_PuuR	kan^R , p15Aori, PuuR under constitutive T7 promoter, 2.9-kb	This study

Table S2: DNA sequences used in this study

#	Name	Sequence	Source
1	T7_RBS	GGTCTCACTATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATAAGAGACC	72978[a]
2	Tag-linker	GGTCTCAGTACTTTAACTTTAAGAAGGAGATATATAAAAGAGACC	72982[a]
3	His-Tag	GGTCTCATAAATGCACCATCACCATCACCATAAGAGACC	72893[a]
4	BBa_B0015	GGTCTCATCGACCAGGCATCAAATAAAACGAAAGCGTTTATAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATGTTAGAGACC	72998[a]
5	PuuR	GGTCTCACATATATGAGTGATGAGGGACTGGCGCCAGGAAAACGCTTGTCGGAAATCCGCCAGCAGCAGGGGCTTTCACAACGTCGTGCCGCCGAACTCTCCGGGCTGACTCACAGTGCTATCAGTACGATAGAACAAGATAAAGTCAGCCCTGCCATCAGTACGCTGCAAAAGCTGCTGAAGGTGTATGGTCTGTCACTCTCGGAATTCTTTTCCGAGCCGGAAAAACCTGATGAGCCGCAGGTCGTCATTAATCAGGACGACTTAATTGAGATGGGTAGTCAGGGTGTGTCAATGAAGCTGGTTCATAACGGTAACCCGAATCGCACGCTGGCGATGATCTTTGAAACGTACCAGCCGGGCACAACCACTGGGGAAAGAATTAAGCATCAGGGTGAGGAAATAGGCACTGTACTGGAAGGTGAAATTGTTCTGACGATTAATGGTCAGGATTACCACCTCGTCGCGGGGCAAAGTTATGCCATTAATACCGGCATCCCGCACAGTTTCAGTAATACGTCGGCAGGTATTTGCCGAATTATCAGCGCCCATACGCCCACCACGTTTTAAGGATCCTCGAAGAGACC	945886[b]
6	eGFP	GGTCTCACCGTCTCAATCTATCTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGATCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAATCGAAGAGACC	72960[a]
7	PuuAp(WT)	GGTCTCACTATTCATTTTTGCAAACTCAATTTAACATTTGACAAACATTTAGTTTGCATACAGATTCGAATGGTGGTCATTATATTTTACGCTTTGGTACAGAGACC	This study
9	TacR(2)	GGTCTCACTATCTGTTGACAGTGGTCATTATATTTTACGCTATAATGTGTGGAGTGGTCATTATTTTACGCGTACAGAGACC	This study
16	TacR(3)	GGTCTCACTATCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAGTGGTCATTATATTTTACGCGTACAGAGACC	This study
12	LR(2)	GGTCTCACTATATAAATTATCTCTGGCGGTGTTGACAGTGGTCATTATTATATTTTACGCGATACTGAGCACGTGGTCATTATATTTTACGCGTACAGAGACC	This study
17	P_{hyb}(3A)	GGTCTCACTATCTGTTGACAGTGGTCATTATATTTTACGCGATACTGAGCACAGTGGTCATTATATTTTACGCTTTGGAGCACGTGGTCATTATATTTTACGCGTACAGAGACC	This study
18	P_{hyb}(1B)	GGTCTCACTATCTGTTGACAATGTTCAATATTTTTTTCAATGATACTGAGCACAGTGGTCATTATATTTTACGCTTTGGAGCACGTGGTCATTATATTTTACGCGTACAGAGACC	This study
20	P_{hyb}(2B)	GGTCTCACTATCTGTTGACAATGTTCAATATTTTTTCAATGATACTGAGCACAATGTTCAATATTTTTTCAATTTTGGAGCACGTGGTCATTATATTTTACGCGTACAGAGACC	This study
22	P_{hyb}(3B)	GGTCTCACTATCTGTTGACAATGTTCAATATTTTTTCAATGATACTGAGCACAATGTTCAATATTTTTTCAATTTTGGAGCACATGTTCAATATTTTTTCAATGTACAGAGACC	This study
19	P_{hyb}(3C)	GGTCTCACTATCTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTACGCTTTGGAGCACGTGGTCATTATATTTTACGCGTACAGAGACC	This study
21	P_{hyb}(3C)	GGTCTCACTATCTGTTGACAATGTTCAATATTTTTTCAATGATACTGAGCACAATGTTCAATATTTTTTCAATTTTGGAGCACGTGGTCATTATATTTTACGCGTACAGAGACC	This study
23	P_{hyb}(3C)	GGTCTCACTATCTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGACTAAATTATCGCCATTTTGGAGCACGTGGACTAAATTATCGCCATGTACAGAGACC	This study

[a] Addgene gene number
[b] NCBI gene number
§Underlined regions are BsaI restriction sites

Table S3: List of PCR primers used in this study

(#)	Primer Name	Sequence 5'-3'
1	TacR(3)_1F	CACCAAGGTCTCACTATCTGTTGACAATTAATCATCGGCTC
2	TacR(3)_2F	CTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAGTGG
3	TacR(3)_1R	TGTGGAGTGGTCATTATATTTTACGCGTACAGAGACCTTGGTG
4	TacR(3)_2R	GCGTAAAATATAATGACCACTCCACACATTATACGAGC
5	P_{hyb}(1B)_2R	GCGTAAAATATAATGACCACGTGCTCCAAAGCGTAAAATATAATGACCACTGTGCTCA
6	P_{hyb}(1B)_R	CACCAAGGTCTCTGTACGCGTAAAATATAATGACCACGTGC
7	P_{hyb}(1B)_2F	CTGTTGACAATGTTCAATATTTTTTCATGATACTGAGCACAGTGGTCATTATATTTTACG
8	P_{hyb}(1B)_F	CACCAAGGTCTCACTATCTGTTGACAATGTTCAATATTTTTTCATGATAC
9	P_{hyb}(1C)_2R	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
10	P_{hyb}(1C)_R	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
11	P_{hyb}(1C)_2F	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
12	P_{hyb}(1C)_F	CACCAAGGTCTCACTATCTGTTGACAGTGGACTAAATTATCGC
13	TacR(2)_2F	CTGTTGACAGTGGTCATTATATTTTACGCTATAATGTGTGGAGTGG
14	TacR(2)_1F	CACCAAGGTCTCACTATCTGTTGACAGTGGTCATTATATTTTACG
15	TacR(2)_2R	GCGTAAAATAATGACCACTCCACACATTATAGCGTAAAATATA
16	TacR(2)_1R	CACCAAGGTCTCTGTACGCGTAAAATAATGACCACTCCACA
17	P_{hyb}(2B)_2R	GCGTAAAATATAATGACCACGTGCTCCAAAATGAAAAAATATTGAACATTGTGCTCA
18	P_{hyb}(2B)_R	CACCAAGGTCTCTGTACGCGTAAAATATAATGACCACGTGC
19	P_{hyb}(2B)_2F	CTGTTGACAATGTTCAATATTTTTTCATGATACTGAGCACAATGTTCAATATTTTTTCATTTTG
20	P_{hyb}(2B)_F	CACCAAGGTCTCACTATCTGTTGACAATGTTCAATATTTTTTCATGATAC
21	P_{hyb}(2C)_2R	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
22	P_{hyb}(2C)_R	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
23	P_{hyb}(2C)_2F	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
24	P_{hyb}(2C)_F	CTGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGTCATTATATTTTA
25	P_{hyb}(3A)_2R	ATGAAAAAATATTGAACATGTGCTCCAAAATGAAAAAATATTGAACATTGTGCTCAGTAT
26	P_{hyb}(3A)_2R	GCGTAAAATATAATGACCACGTGCTCCAAAGCGTAAAATATAATGACCACTGTGCTCAGTATC
27	P_{hyb}(3A)_R	CACCAAGGTCTCTGTACGCGTAAAATATAATGACCACGTGC
28	P_{hyb}(3A)_F	CACCAAGGTCTCACTATCTGTTGACAGTGGTCATTATATTTTACGC
29	P_{hyb}(3B)_2F	GTTGACAGTGGTCATTATATTTTACGCGATACTGAGCACAGTGGTCATTATATTTT
30	P_{hyb}(3B)_R	CACCAAGGTCTCTGTACATGAAAAAATATTGAACATGTGCTCCAA
31	P_{hyb}(3B)_2F	TGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGACTAAATTATCGCCATTTT
32	P_{hyb}(3B)_F	CACCAAGGTCTCACTATCTGTTGACAGTGGACTAAATTATCGC
33	P_{hyb}(3C)_2R	ATGGCGATAATTTAGTCCACGTGCTCCAAAATGGCGATAATTTAGTCCACTGT
34	P_{hyb}(3C)_R	CACCAAGGTCTCTGTACATGGCGATAATTTAGTCCACGTG
35	P_{hyb}(3C)_2F	TGTTGACAGTGGACTAAATTATCGCCATGATACTGAGCACAGTGGACTAAATTATCGCCATTTT
36	P_{hyb}(3C)_F	CACCAAGGTCTCACTATCTGTTGACAGTGGACTAAATTATCGC
37	PuuAp_1F	CACCAAGGTCTCACTATTCATTTTTGCAAACTCAATTTAACATTTGAC
38	PuuAp_2F	TCATTTTTGCAAACTCAATTTAACATTTGACAAACATTTAGTTTGCATACAGATTC
39	PuuAp_1R	CACCAAGGTCTCTGTACCAAAGCGTAAAATATAATGACCACCATT
40	PuuAp_2R	CAAAGCGTAAAATATAATGACCACCATTCGAATCTGTATGCAAACTAAATGTTTGTCAA
45	pFab4876_frwd_BsaI	AACGTCTCAATCTCTATAGAGACCCAATGTAGCACCTGAAGTCAGCC
46	pFab4876_rev_BsaI	GGGTCTCTATAGAGATTGAGACGTTTGAAGAGATAAATTGCACTGAAATCTAGAA
47	pFab4876_frwd_2BsaI	CGGTCTCATGTTTTAGAGAGACGTTACTAGTGCTTGGATTCTCACC
48	pFab4876_rev_2BsaI	CGTCTCTCTAAAACATGAGACCGACAACTTATATCGTATGGGGCT
49	PuuR_F	CACCAAGGTCTCACATATGATGAGTGATGAGGGACTGGC
50	PuuR_R	CACCAAGGTCTCTTCGAGGATCCTTAAAACGTGGTGGGCGTATG
51	pFAB_delphos_FRWD	GGATCGGTTGTCGAGTAAGGATC
52	pFAB_delphos_REV	TCATGCACAGGAGACTTTCTAATG
53	L440	AGCGAGTCAGTGAGCGAG
54	pBp_ECOR1	AAAAATAGGCGTATCACGAGGC

Table S4: Volumes and thermocycle conditions for PCR

Colony PCR

Phusion Master Mix	16.8 µL
Forward Primer	1.0 µL
Reverse Primer	1.0 µL
Template (colony dissolved in 20µL H_2O)	1 µL
Phusion	0.2 µL
Total	20.0 µL

Template Conditions

Phusion Master Mix	43.5 µL
Forward Primer	2.5 µL
Reverse Primer	2.5 µL
Template (colony dissolved in 20µL H_2O)	1.0 µL
Phusion	0.5 µL
Total	50.0 µL

Thermocycler Conditions

Step	Number of Cycles	Temperature	Time
Initial Denaturation	1	98 ˚C	30 s
Denaturation	35	98 ˚C	10 s
Annealing		45 ˚C -72 °C	20 s
Extension		72 ˚C	30 s
Final Extension	1	72 ˚C	10-12 min
hold	1	4 ˚C-10 ˚C	∞

Table S5: Golden Gate Conditions

Temperature	Time	Number of cycles
37 ˚C	5 min	15-30
16 ˚C	10 min	
50 ˚C	5 min	1
80 ˚C	5 min	1

Table S6: PuuR binding regions for our synthetic promoters

Binding Region	Sequence	Source
A	GTGGTCATTATATTTTACGC	[7-9]
B	ATGTTCAATATTTTTTCAAT	[7-9]
C	GTGGACTAAATTATCGCCAT	[10]

Table S7. Costs of reagents and materials for producing in-house cell-free extract

Chemical	Mass	Price
$Mg(OAc)_2$	50g	22.30
K(OAc)	1kg	223.00
Tris (OAc)	500g	149.0
IPTG	5g	66.00
DTT	5g	24.00
tRNA	100mg	219.00
Folinic Acid	100mg	142.00
NTPs	4x200µL	297.00
Oxalic Acid	5g	38.90
NAD	1g	124.00
PEP	25mg	227.00
Acetal CoA	25mg	129.00
K(Glu)	500g	249.00
$Mg(Glu)_2$	250g	83.00
NH4(Glu)	100g	255.28
Spermidine	5g	197.00
HEPES	25g	55.00
Cell-Free 20 aa mix	1mlx100mM of each amino acid	372.00
LB-Media	1L*4	43.00
Total		3044.20
Price per Reaction		1.52

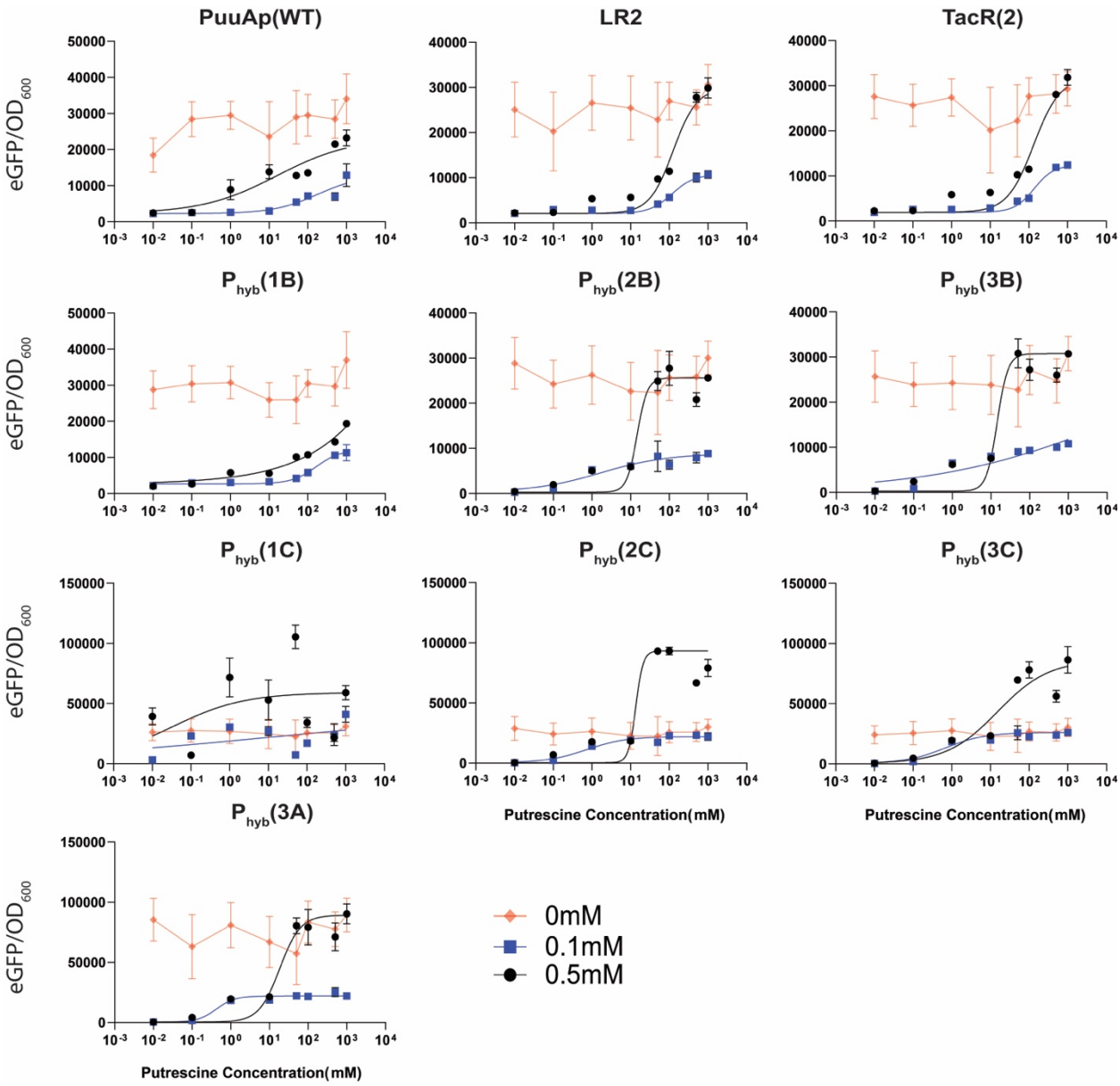


Figure S1 – Repression dynamics. Repressor expression was induced with IPTG at two concentrations 0.1 and 0.5 mM. The putrescine was exogenously added at a final concentration of (0, 0.01, 0.1, 1, 10, 50, 100, 500, 1000 mM). Each point represents eGFP fluorescent output measured at the end of a 16 h incubation period and normalized to their OD. Semi-log curves were fitted by Prism 9.0 using a 4-parameter non-linear regression function. Fluorescent measurements were done in triplicates and error bars are representing one standard deviation.

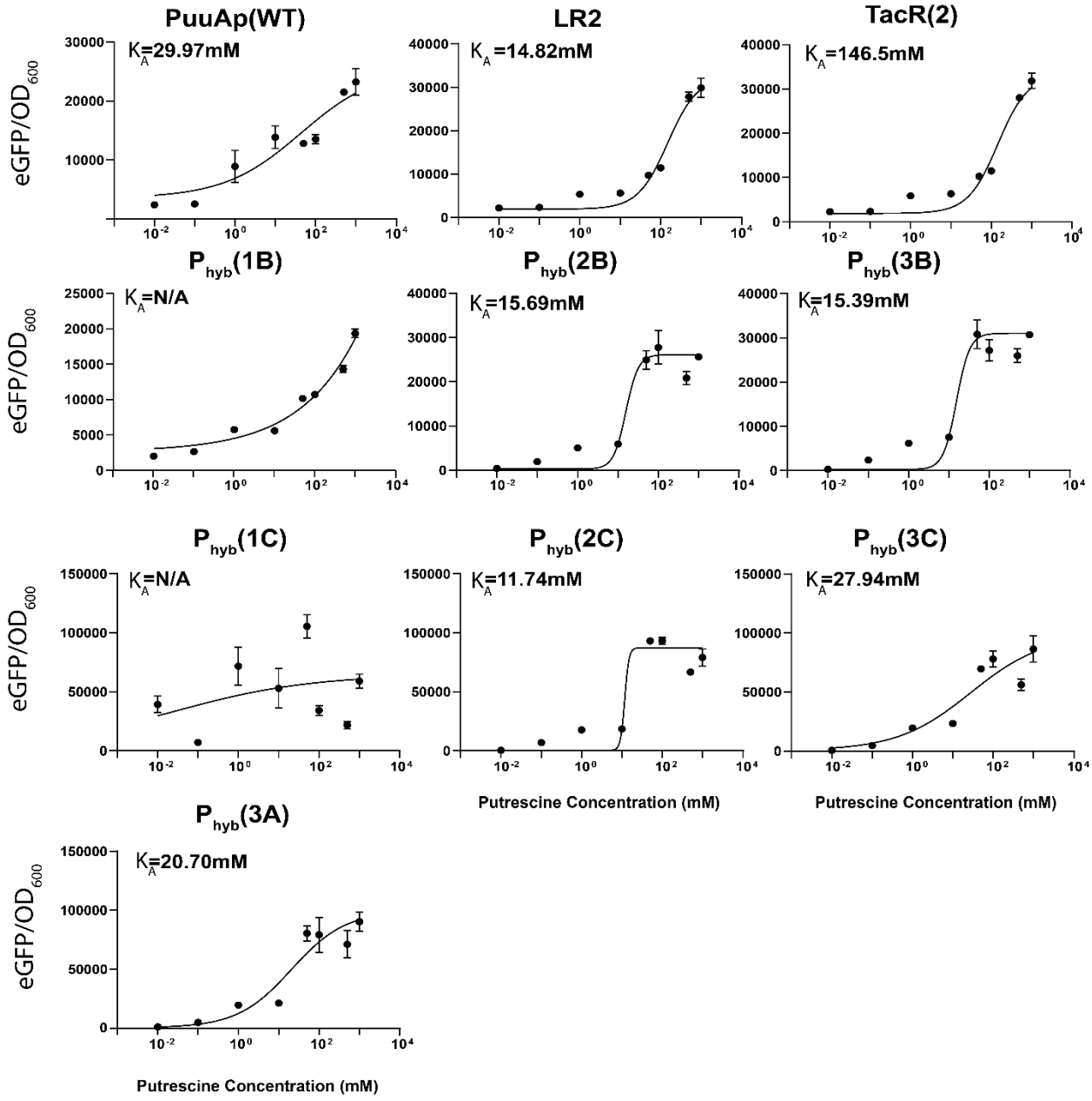


Figure S2 - Dose-response curves for our synthetic promoters. Each promoter was induced with 0.5 mM IPTG and with putrescine at a final concentration of 0, 0.01, 0.1, 1, 10, 50, 100, 500, 1000 mM. Each point represents an eGFP fluorescent output measured at the end of a 16 h incubation period and normalized to their OD. Semi-log curves were fitted by Prism 9.0 using a 4-parameter non-linear regression to determine the K_A. Fluorescent measurements were done in triplicates and error bars are representing one standard deviation.

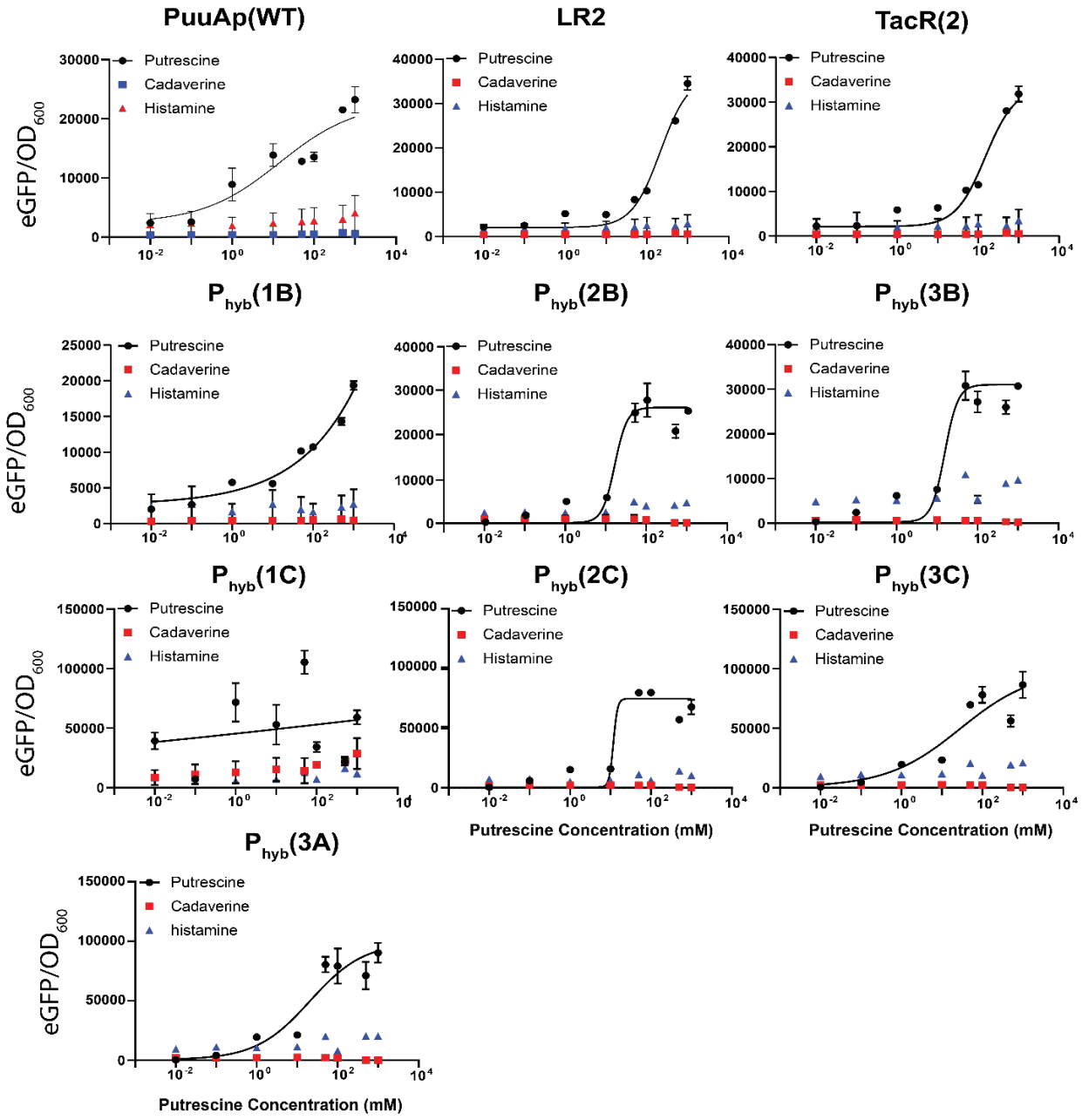


Figure S3 – Specificity testing for our synthetic promoters. Each promoter was induced with 0.5 mM IPTG and different concentrations of putrescine (black) and other chemically similar biogenic amines cadaverine (red) and histamine (blue) at a final concentration of 0, 0.01, 0.1, 1, 10, 50, 100, 500, 1000 mM Each point represents eGFP fluorescent output measured at the end of a 16 h incubation period and normalized to their OD. Semi-log curves were fitted by Prism 9.0 using a 4-parameter non-linear regression to determine the K_A. Fluorescent measurements were done in triplicates and error bars are representing one standard deviation.

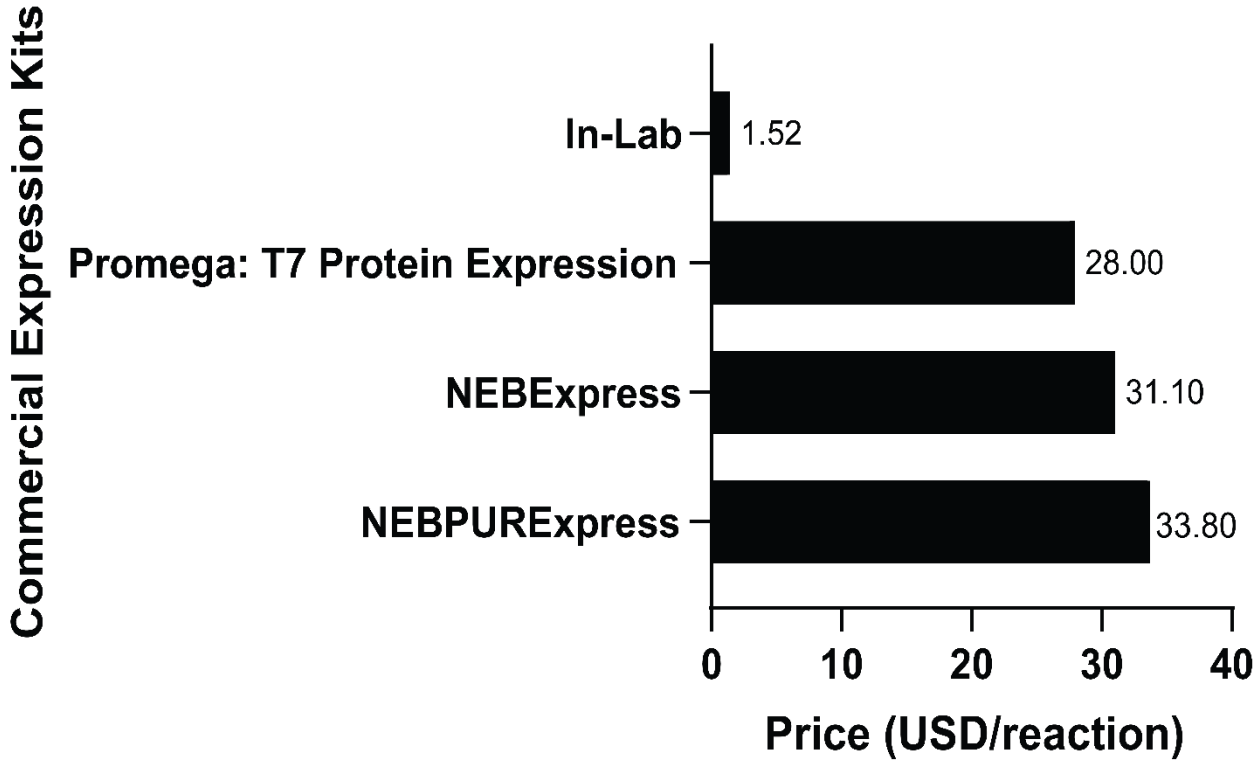


Figure S4 - Cost comparison of our cell-free extract with other commercial kits. The bar graph shows the price per reaction for each cell-free crude kit.

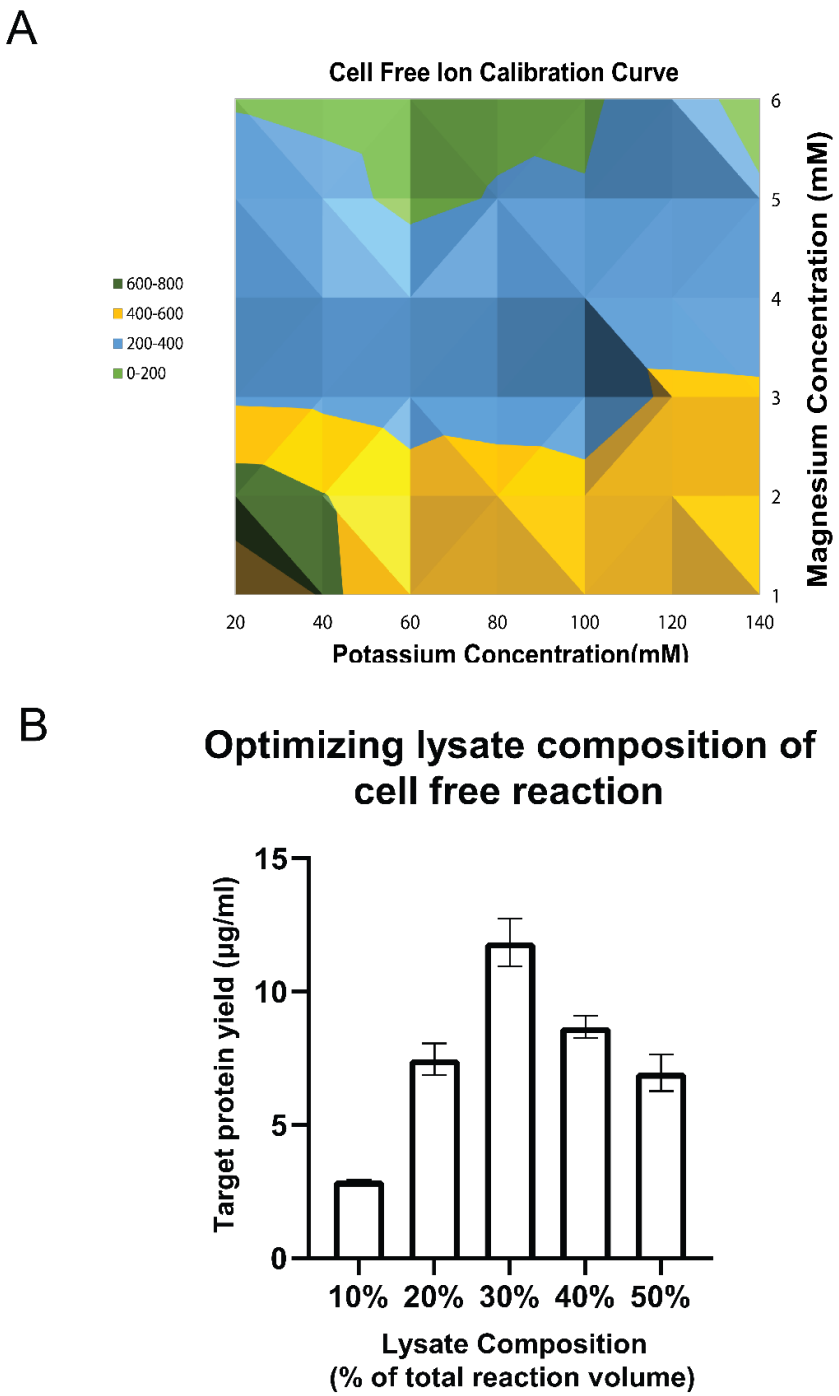


Figure S5 – Optimization of our in-house cell-free system. A) K^+ and Mg^{+2} ions were screened against a lysate with a eGFP protein concentration of 15 µg/mL as the optimal concentration for the expression of eGFP. All cell free reactions were performed in triplicates in 15 µL volumes. Endpoint fluorescence of each reaction was used to generate contour plot. eGFP fluorescence shows maximum at 2 mM of Mg^{+2} and 20 mM of K^+. B) Optimization of eGFP protein yield by changing lysate composition. Error bars show one standard deviation for three independent biological replicates.

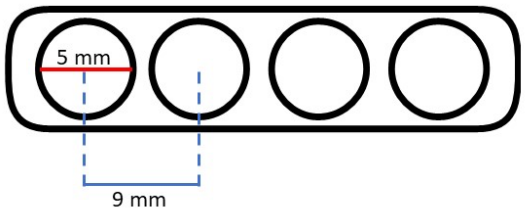


Figure S6 – Paper disc device for assessing meat spoilage. Four 5 mm discs were fabricated 9 mm apart to match the well-to-well dimensions on the well-plate.

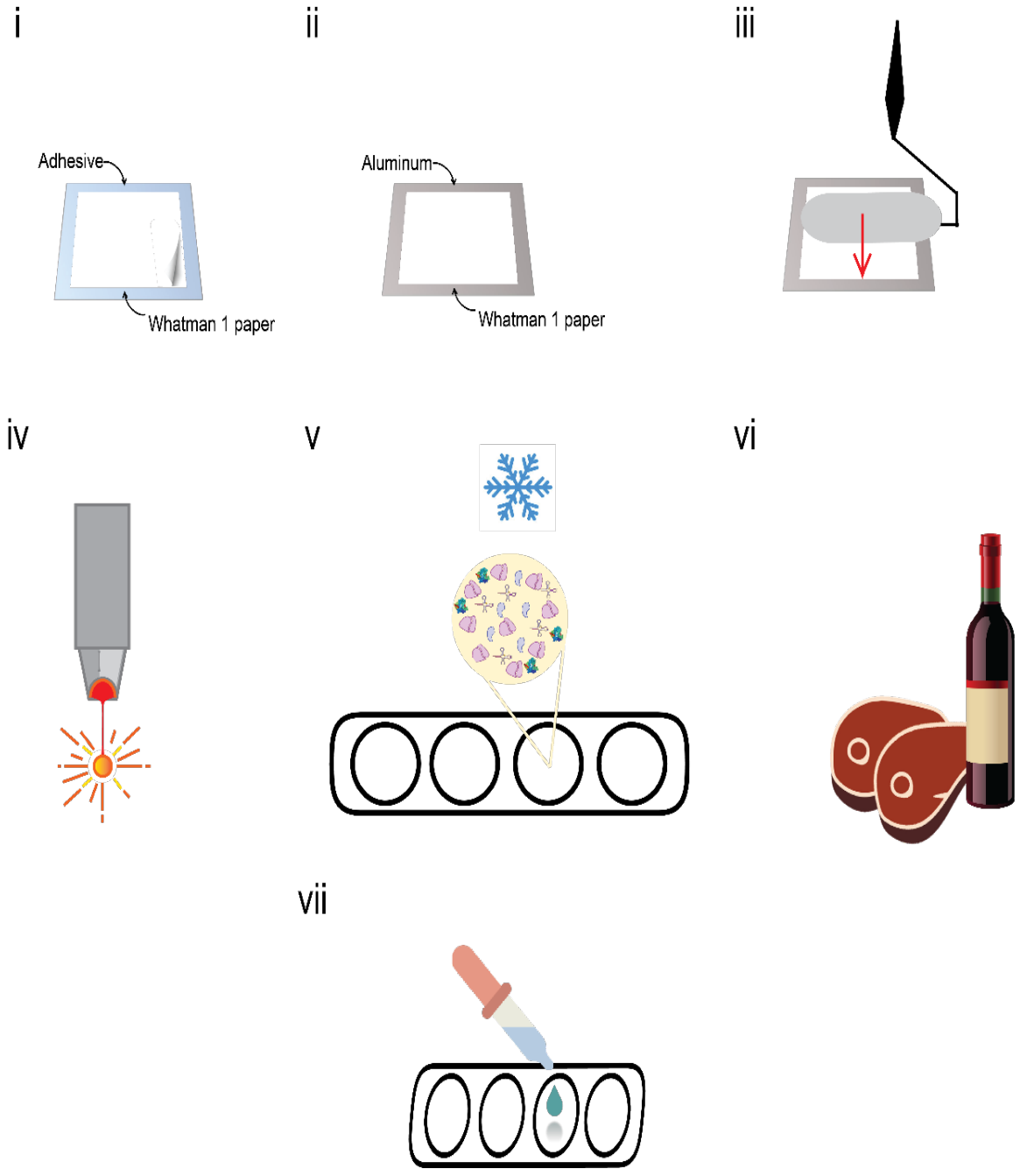
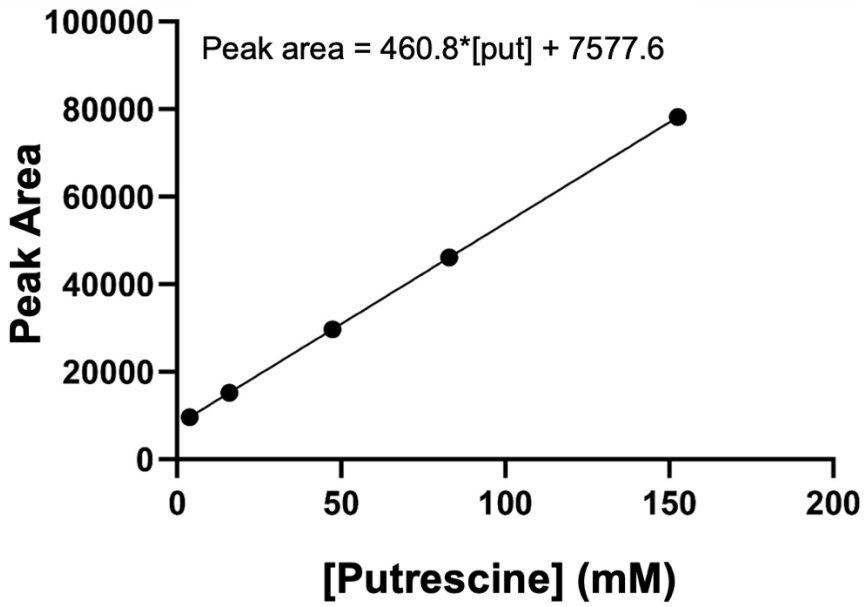


Figure S7 – **Paper device fabrication and sample testing.** Step (i): An adhesive (20cmx20cm) is transferred to *Whatman Grade 1 Chr* paper and then gently peeled off. (ii) Whatman paper is transferred onto aluminum sheet (20cmx20cm). (iii) The foil-backed paper was fed through a manual cold-roll laminator. (iv) The paper aluminum substrate is used for laser cutting paper microfluidic device. (v) Cell free reactions are lyophilized on the paper discs. (vi) Samples are prepared for spoilage assessment. (vii) Food samples are loaded onto the discs to rehydrate and initiate the cell free reactions to yield an eGFP output.

A



B

	Storage Temperature		
Detection System	-20 °C	4 °C	20 °C (room)
HPLC	0 mM	0 mM	19.8 ± 5.2 mM
Biosensor	17.2 mM	83.4 mM	322 mM

Figure S8 – Comparing our cell-free putrescine biosensor with LC-MS. (A) Calibration curve showing the relation between putrescine standards with peak area obtained from the chromatograms. (B) Beef extracts stored at -20 °C, 4 °C, and RT on day 4 (after 4 h) were loaded into a HPLC-MS and the peak areas were converted into putrescine concentration(s) using the calibration curve in (A). These results were compared to putrescine content measured with our biosensor. Samples stored under -20 °C and 4 °C conditions did not show any peaks.

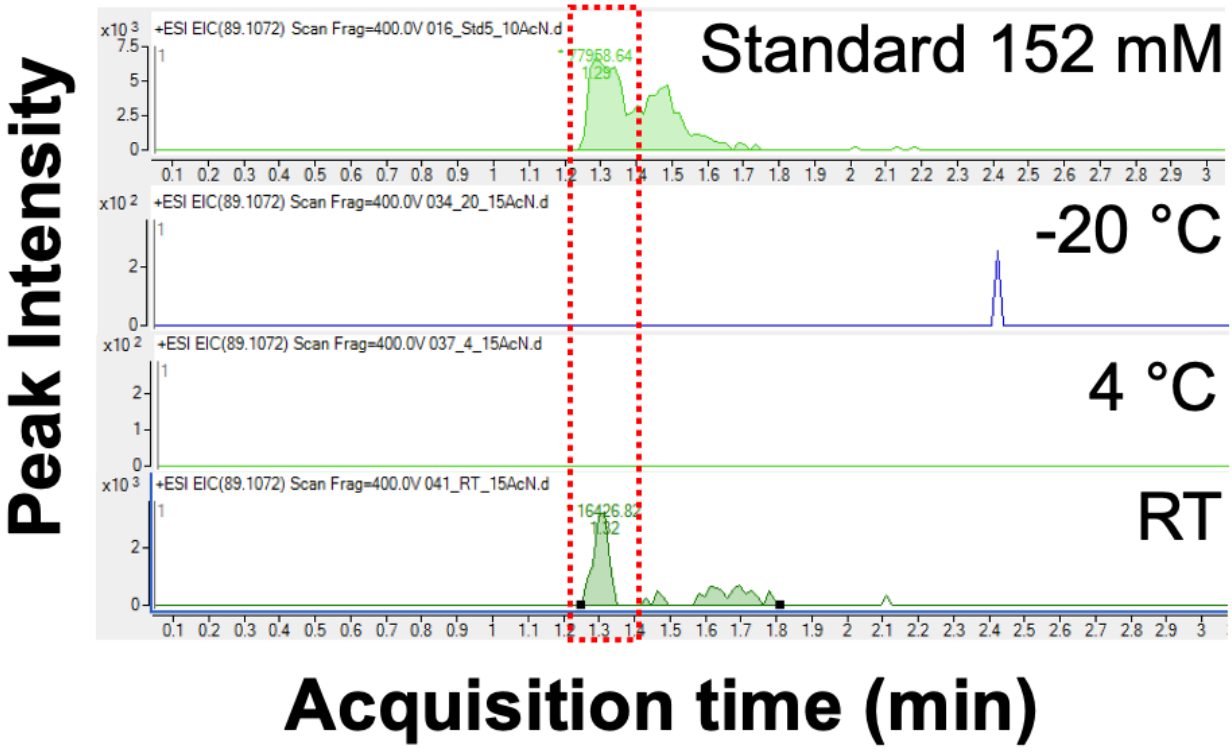


Figure S9 – HPLC-MS chromatograms of a putrescine standard and beef samples stored at three different temperatures (-20 °C, 4 °C, and RT). Extracted chromatograms for putrescine was at m/z = 89.107 with an elution time 1.3 min (see dotted red box). Values above the peaks is the peak area. Three meat samples were measured for putrescine content at each temperature (-20 °C, 4 °C, and room temperature).

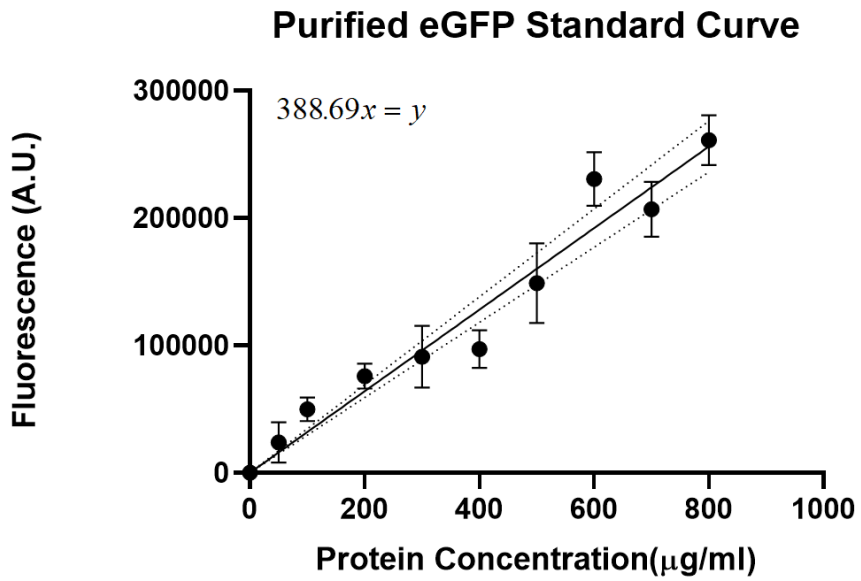


Figure S10 – eGFP calibration curve. eGFP protein was expressed and purified according to Supplementary Protocol S4. This calibration curve was used to convert fluorescence into eGFP yield (μg/mL). The error bars represent one standard deviation for three biological replicates.

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